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Group I metabotropic glutamate receptors stimulate the activity of poly(ADP-ribose) polymerase in mammalian mGlu1-transfected cells and in cortical cell cultures

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Abstract

Group I metabotropic glutamate (mGlu) receptors (i.e. mGlu1 and mGlu5) coupled to phospholipase C have been widely investigated for their possible role in excitotoxic and post-ischemic neuronal death. Recently, phospholipase C has been shown to directly stimulate the activity of poly(ADP-ribose) polymerase (PARP), a nuclear enzyme involved in DNA repair that has been proposed to play a key role in necrotic cell death. In this study, we investigated whether the stimulation of group I mGlu receptors leads to an increase in PARP activity, as detected by flow cytometry, immunodot blot and immunocytochemistry, both in baby hamster kidney cells transfected with mGlu1a or mGlu5a receptors and in cultured cortical cells. Our results show that the group I mGlu receptor agonist DHPG elicited a significant increase in PARP activity that was completely abolished by the administration of the mGlu1 antagonist 3-MATIDA and partially prevented, in cortical neurons, by the mGlu5 antagonist MPEP. To evaluate whether this pathway is involved in post-ischemic neuronal death, we used a sublethal model of oxygen-glucose deprivation in mixed cortical cell cultures. DHPG exacerbated neuronal death, and this effect was significantly prevented by the application of the PARP inhibitor DPQ. This novel pathway may contribute to the effects of mGlu1 receptors in the mechanisms leading to post-ischemic neuronal death.

Keywords: Metabotropic glutamate receptors; Phospholipase C; Poly(ADP-ribose) polymerase; Oxygen-glucose deprivation; 3-MATIDA

1. Introduction

Group I metabotropic glutamate (mGlu) receptors, including mGlu1 and mGlu5 receptors, have been extensively investigated in the past few years for their possible involvement in numerous physiological and pathological states in the brain, including synaptic plasticity, long-term potentiation and depression, apoptosis and neurodegeneration following cerebral ischemia (Bordi and Ugolini, 1999; Bruno et al., 2001; Conn and Pin, 1997; Pellegrini-Giampietro, 2003; Schoepp, 2001). mGlu1 and mGlu5 receptors are thought to couple through Gq-type G-proteins to phospholipase C (PLC)-β, which results in intracellular Ca^{2+} mobilization via inositol (1,4,5)-trisphosphate (IP_3) and ryanodine receptors (Fagni et al., 2000; Stefani et al., 1996). Group I mGlu receptors have also been implicated in a variety of alternative intracellular transduction pathways, including adenylyl cyclase (Aramori and

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Nakanishi, 1992), tyrosine kinase (Heuss et al., 1999), mitogen-activated protein kinase (Calabresi et al., 2001) and phosphoinositide 3-kinase (Rong et al., 2003).

Poly(ADP-ribosyl)ation is a post-translational enzymatic modification involved in numerous cellular processes including DNA repair, genomic integrity and cell death (Chiarugi, 2002; Herceg and Wang, 2001; Smith, 2001). It is catalyzed by poly(ADP-ribose) polymerases (PARPs), a growing family of enzymes that convert NAD into long branched polymers (up to 200 units) of poly(ADP-ribose) (PAR), which are then attached to a large number of acceptor nuclear proteins (including PARP itself). Overactivation of PARP-1, the most studied member of this family, has been associated with the pathogenesis of numerous disorders in the CNS, including excitotoxicity and ischemic injury. A presumed cascade of glutamate activating NOS, leading to excessive formation of NO and DNA damage and then to overactivation of PARP-1, has been proposed to play a "suicidal role" due to the marked depletion of NAD and ATP tissue stores and the disruption of oxidative metabolism (Ha and Snyder, 2000; Herceg and Wang, 2001; Szabó and Dawson, 1998). Accordingly, pharmacological PARP inhibition or targeted deletion of the PARP-1 gene provides impressive and unparalleled protection against neuronal damage in several animal models of excitotoxicity (Mandir et al., 2000; Moroni et al., 2001; Pieper et al., 1999). More recent findings also suggest a "transcriptional hypothesis" for the neurotoxic effects of PARP-1, in which the formation of PAR regulates the gene expression of neuroactive proteins such as iNOS, IL-1β, COX-2, TNF-α and APP (Chiarugi, 2002).

Activation of PARP is typically initiated by stressful stimuli that damage DNA, but recent reports suggest that it can also be induced by physiological activity, in the absence of DNA damage (Cohen-Armon et al., 2004). In particular, it has been demonstrated that PARP can be rapidly activated by stimulation of PLC and IP_3-evoked Ca^{2+} mobilization (Homburg et al., 2000). Hence, we decided to investigate whether the stimulation of group I mGlu receptors could promote the activation of PARP in baby hamster kidney (BHK) transfected with mGlu1 or mGlu5 receptors and in cortical cell cultures. Moreover, we examined whether this mechanism could play a role in an in vitro model of cerebral ischemia.

2. Methods

2.1. Materials

3-Amino-thiophene-dicarboxylic acid (3-MATIDA) was synthesized as previously described (Moroni et al., 2002). (*RS*)-3,5-dihydroxyphenylglycine (DHPG) and 2-methyl-6-(phenylethynyl)pyridine hydrochloride (MPEP) were purchased from Tocris Bioscience (Northpoint, UK), whereas 3,4-dihydro-5-[4-1(1-piperidinyl)buthoxy]-1(2H)-isoquinolinone (DPQ), the polyclonal rabbit antibody (LP98-10) and the monoclonal mouse antibody (10H) directed against PAR were from Alexis Corporation (Vinci, Italy). Lactate dehydrogenase (LDH) activity was quantified using the Cytotoxicity Detection Kit (LDH) from Roche Diagnostics (Monza, MI, Italy). The anti-glial fibrillary acidic protein (GFAP) fluoresceine isothiocyanate (FITC)-conjugated antibody and the phycoerythrin-conjugated goat anti-rabbit IgG were purchased from Molecular Probes Europe (Leiden, The Netherlands). Tissue culture reagents were obtained from GIBCO-BRL (Milan, Italy) and ICN Pharmaceuticals (Opera, Milan, Italy). All other reagents were of analytical grade and obtained from Merck (Darmstadt, Germany).

2.2. Transfected cell cultures

Baby hamster kidney (BHK) cells stably transfected with rat mGlu1a or mGlu5a receptors (see Moroni et al., 2002) were provided by Dr. C. Thomsen (Novo Nordisk, Denmark) and cultured in Dulbecco's modified Eagle's medium supplemented with 5% dialyzed fetal bovine serum, 2 mM glutamine, 0.05 mg/ml gentamicin and 0.1 mg/ml neomycin in a humidified atmosphere (95% air/5% CO_2) at 37 °C. In addition, the incubation medium of the transfected cells was supplemented with G-418 and methotrexate. Cells were maintained in a humidified atmosphere (95% air/5% CO_2) at 37 °C and subcultured (every second day) using 0.05% trypsin/EDTA.

2.3. Murine cortical cell cultures

Cultures of mixed cortical cells containing both neuronal and glial elements were prepared as previously described in detail (Pellegrini-Giampietro et al., 1999a,b) and used at 14–15 days in vitro (DIV).

2.4. Measurement of PARP activity by flow cytometry

PARP activity was evaluated in BHK transfected cells and in murine cortical cells by cytofluorimetric measurement of PAR formation according to Affar et al. (1999), with minor modifications. Following DHPG exposure (30 min for transfected BHK cells, 60 min for cortical cells), BHK cell cultures were resuspended and fixed in 4% paraformaldehyde, whereas cortical cell cultures were detached using 0.05% trypsin for 5 min at 37 °C in PBS, washed with cold PBS and then fixed with 4% paraformaldehyde at room temperature. Fixed cells were permeabilized with 0.2% NP-40, washed with PBS, saturated with PBS-MT (PBS containing 5% non-fat powdered milk and 0.1% Tween 20) for 1 h and then incubated overnight at 4 °C with anti-PAR (LP98-10, 1:100) and, in the case of mixed cortical cell cultures, with

FITC-conjugated anti-GFAP (1:100) antibodies diluted in PBS-MT. After several washes with PBS-MT, cells were incubated with a phycoerythrin-conjugated anti-rabbit IgG (1:50) for 30 min. The cell suspension (approximately 10,000 cells per analysis) was analyzed using a flow cytometer (Coulter XL, Coulter Cytometry, Hialeah, FL, USA). In the case of mixed cortical cell suspensions, containing both neurons and astrocytes, neuron-related events (PAR-positive neurons) were sorted by subtracting FITC-labeled cells (GFAP-positive astrocytes) from phycoerythrin-positive cells by means of appropriate electronic gates.

2.5. Measurement of PARP activity by immunodot blot

In BHK transfected cells, PARP activity was also evaluated by immunodot blot analysis. Briefly, proteins were extracted, diluted in NaOH/EDTA and 2 μl of each sample was loaded onto Hybond N+ membranes. After washing with NaOH and an incubation with PBS-MT, membranes were exposed overnight to a monoclonal antibody to PAR (10H, 1:250). An HRP-conjugated secondary antibody was then added and membrane was analyzed by a chemiluminescence method (ECL-Plus, Amersham Biosciences).

2.6. Immunocytochemistry

In mixed cortical cultures, PARP activity was also evaluated by immunocytochemistry. Mixed cultures containing both neurons and glia were washed with cold PBS and then fixed in 100% methanol. Fixed cells were washed again with cold PBS, saturated with PBS-MT for 1 h and then exposed for 90 min at 37 °C to a monoclonal antibody to PAR diluted in PBS-MT (10H, 1:100). After washing with PBS-MT, cells were incubated 60 min at 37 °C with a fluorescent secondary antibody (Alexa 488, Molecular Probes, 1:50) and then observed under fluorescence optics.

2.7. Oxygen-glucose deprivation (OGD) in cortical cell cultures

Cultures of mixed cortical cells were exposed to oxygen-glucose deprivation (OGD) as previously described in detail (Pellegrini-Giampietro et al., 1999a,b). Briefly, culture medium was replaced by a glucose-free balanced salt solution saturated with 95% N_2/5% CO_2 and heated to 37 °C. Multiwells were then sealed into an airtight incubation chamber equipped with inlet and outlet valves and 95% N_2/5% CO_2 was blown through the chamber for 10 min to ensure maximal removal of oxygen. The chamber was then sealed and placed into the incubator at 37 °C for 60 min. OGD was terminated by removing the cultures from the chamber, replacing the exposure solution with oxygenated medium and returning the multiwells to the incubator under normoxic conditions. The extent of neuronal death was assessed 24 h later. OGD-induced cell injury was quantitatively evaluated by measuring the amount of LDH released from injured cells into culture media 24 h following exposure to OGD, as previously described (Pellegrini-Giampietro et al., 1999a,b). The LDH level corresponding to complete neuronal death (with no glial death) was determined for each experiment by assaying sister cultures exposed to 1 mM glutamate for 24 h. Background LDH release was determined in control cultures not exposed to OGD and subtracted from all experimental values. The resulting value correlated linearly with the degree of cell loss estimated by observation of cultures under phase-contrast microscopy or under bright-field optics following 5 min incubation with 0.4% trypan blue, which stains debris and nonviable cells.

3. Results

3.1. Studies in BHK cells stably transfected with mGlu1a and mGlu5a receptors

Poly(ADP-ribosyl)ation evoked by receptor stimulation was evaluated in BHK cells stably transfected with mGlu1a and mGlu5a receptors by measuring the formation of PAR, the product of PARP activity, with a flow-cytometric assay (Fig. 1). In control cells, only a background peak at low levels of relative PAR fluorescence intensity was observed. Thirty min after the exposure, DHPG (50–100 μM) promoted the appearance of a second peak at higher levels of relative PAR fluorescence intensity, indicative of increased PARP activity and increased formation of PAR. The effect of DHPG was approximately 80% of that observed with 300 μM H_2O_2 (15 min) and was almost completely abolished by co-application of the selective mGlu1 receptor antagonist 3-MATIDA (100 μM, Fig. 2A). The selective mGlu5 receptor antagonist MPEP (1 μM, used as a negative control) had no effect. In wild-type non-transfected BHK cells and in BHK cells transfected with mGlu5a receptors, the formation of PAR could be elicited only by exposure to H_2O_2, but not by DHPG (Fig. 2B).

PARP activity in BHK transfected cells was also measured by immunodot blot analysis. Similarly to what observed with flow cytometry, both the positive control H_2O_2 and, to a somewhat lesser degree, DHPG (100 μM, 30 min) significantly increased the formation of PAR. The DHPG-evoked increase was dramatically reduced by the mGlu1 antagonist 3-MATIDA (Fig. 3).

3.2. Studies in murine cortical cell cultures

Poly(ADP-ribosyl)ation evoked by stimulation of mGlu receptors was evaluated in mixed cortical cells

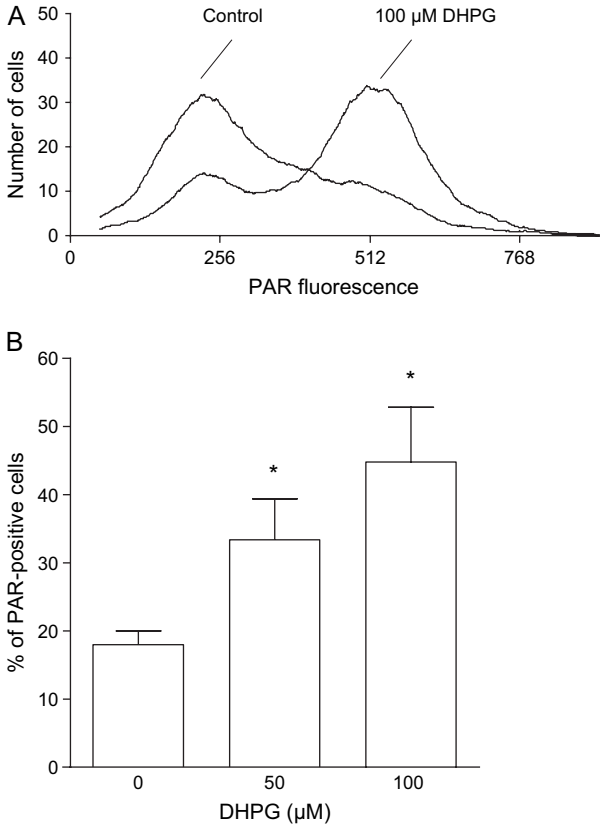


Fig. 1. PARP activation following DHPG exposure in BHK cells transfected with mGlu1a receptors. Transfected BHK cells were exposed to 50 or 100 μM DHPG and after 30 min they were fixed, labeled with a polyclonal antibody directed against PAR, the product of PARP, and processed for flow cytometry analysis. (A) The graph plots the relative number of cells displaying increasing levels of PAR fluorescence and is representative of five analyses. DHPG exposure elicited the formation of a peak at high levels of relative fluorescence intensity, indicative of the neosynthesis of PAR. (B) Percentage of PAR-positive cells in the entire cell population, as calculated by counting the relative number of cells detected at channels for high levels of PAR fluorescence (>350). DHPG dose-dependently increased the formation of PAR. Results are the mean ± SEM of five experiments. *$P < 0.05$ vs. untreated control (ANOVA + Tukey's *w*-test).

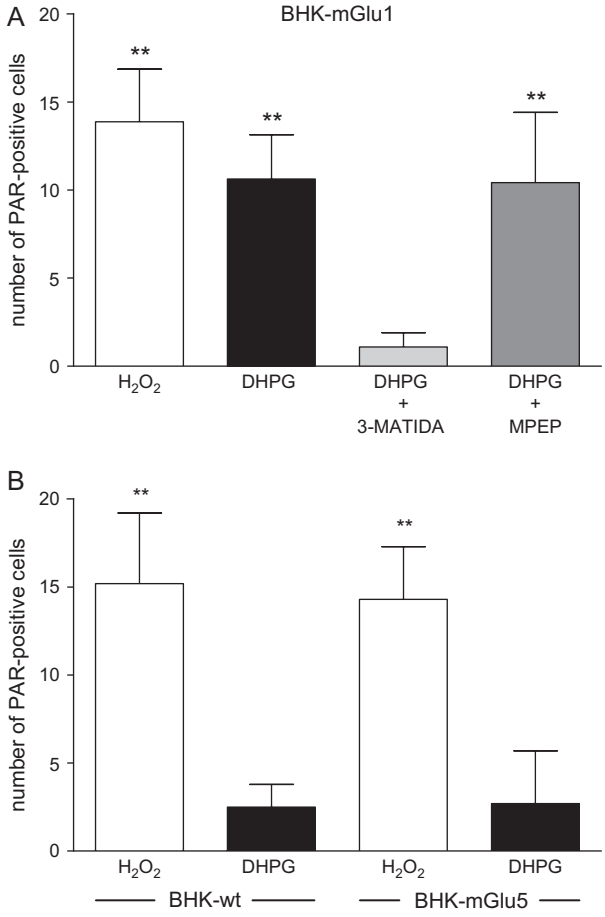


Fig. 2. PARP activation in wild-type and transfected BHK cells: effects of group I mGlu receptor antagonists. Wild-type (wt) BHK cells and BHK cells transfected with mGlu1a or mGlu5a receptors were exposed to 300 μM H_2O_2 for 15 min or 100 μM DHPG for 30 min and then fixed, labeled with an antibody directed against PAR, the product of PARP, and processed for flow cytometry analysis. (A) In BHK cells transfected with mGlu1a receptors, 3-MATIDA (100 μM) or MPEP (1 μM) were added to the incubation medium 15 min prior to DHPG. Data are expressed as percentage of PAR-positive cells in the entire cell population. The DHPG-induced formation of PAR was blocked by 3-MATIDA but not by MPEP. Results are the mean ± SEM of at least four experiments. **$P < 0.01$ vs. untreated cells (ANOVA + Tukey's *w*-test). (B) In wild-type or mGlu5a-transfected BHK cells, H_2O_2 but not DHPG increased the formation of PAR. Results are the mean ± SEM of at least three experiments. **$P < 0.01$ vs. untreated cells (ANOVA + Tukey's *w*-test).

using immunocytochemistry (Fig. 4) and flow cytometry (Fig. 5). H_2O_2 (500 μM for 15 min) induced a dramatic increase in PAR immunoreactivity in neuronal cells, but not in the underlying glial cell layer (Fig. 4B). A much weaker, but clearly evident, appearance of PAR-positive staining was observed in neuronal cells following application of the group I mGlu receptor agonist DHPG (100 μM for 60 min, Fig. 4C), that was completely prevented by the addition of 100 μM 3-MATIDA (Fig. 4D). This result was confirmed by flow cytometry, in which the exposure of neuronal cells to 100 μM DHPG for 60 min elicited the formation of a peak at higher levels of relative PAR fluorescence intensity, indicating the neosynthesis of PAR (Fig. 5A). The mGlu1 receptor selective antagonist 3-MATIDA (100 μM) completely abolished this effect, whereas the mGlu5 receptor selective antagonist MPEP (1 μM) reduced the DHPG-evoked increased formation of PAR by approximately 60% (Fig. 5B).

3.3. Studies in a cortical cell model of sublethal oxygen-glucose deprivation

In order to evaluate the functional significance of the mGlu-PARP pathway in post-ischemic neuronal death, we utilized murine cortical cell cultures exposed to sublethal OGD. We selected this particular OGD model in order to isolate the potentially toxic effects of DHPG

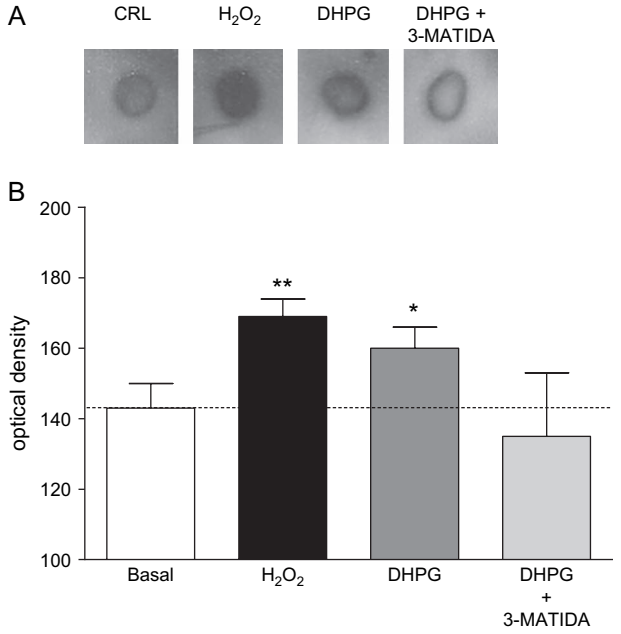


Fig. 3. Immunodot blot analysis of PAR formation in BHK cells transfected with mGlu1a receptors. Cells were treated as indicated in Fig. 2, then proteins were extracted, loaded onto Hybond N+ membranes and incubated overnight with a monoclonal PAR antibody. Membranes were analyzed by chemiluminescence and quantitated with the aid of a computer-assisted software. (A) Sample optic density increased in the positive control (H_2O_2) and in DHPG-treated cells. (B) Quantitative analysis of optic densities showing that 3-MATIDA reduced the increase in PAR formation induced by DHPG. Each bar represents the mean ± SEM of four experiments. $^{**}P < 0.01$ and $^{*}P < 0.05$ vs. basal (ANOVA + Tukey's *w*-test).

and the component of OGD injury evoked by DHPG. As previously described (Pellegrini-Giampietro et al., 1999a), the extent of neuronal death in this system increased with the duration of exposure to OGD (Fig. 6A). Hence, exposures <30 min produced little neuronal injury when observed 24 h later, whereas exposure durations >40 min induced increasing levels of neuronal damage. As a sublethal OGD we selected an exposure duration of 40 min, which caused a release of LDH in the medium that was approximately 40% of that observed by exposing the cultures to 1 mM glutamate for 24 h. The addition of 100 μM DHPG to the medium during OGD and the subsequent 24 h recovery period significantly exacerbated neuronal damage (Fig. 6B). The PARP inhibitor DPQ (at 10 μM) reduced the release of LDH induced by sublethal OGD in a modest, non-significant, fashion but completely blocked the effect of DHPG (Fig. 6B).

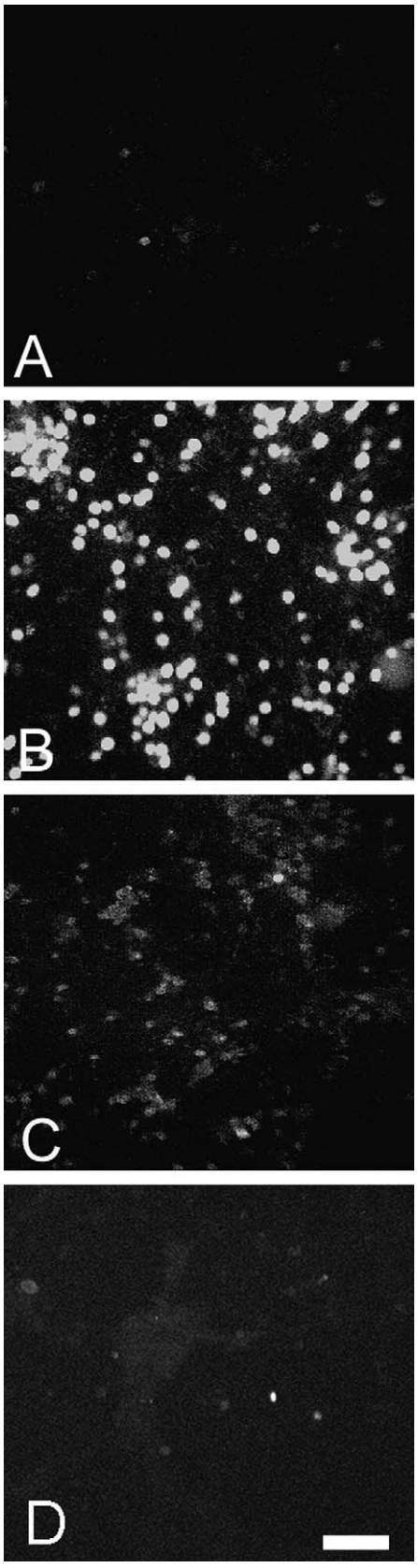
4. Discussion

Our results show that stimulation of group I mGlu receptors elicits a significant increase in PARP activity in both BHK cells transfected with mGlu1 receptors and in murine cortical cell cultures. This was demonstrated by means of flow cytometry, immunodot blot analysis (in BHK cells) and immunocytochemistry (in neurons) using antibodies directed against PAR, the product of PARP activity. In addition, we show that mGlu receptor-stimulated PARP activation may play a role in an in vitro model of sublethal ischemia.

It has been clearly and repeatedly demonstrated that glutamate receptors of the NMDA but not of the AMPA type are able to activate PARP in neurons (Mandir et al., 2000; Meli et al., 2004), possibly via the formation of nitric oxide and peroxynitrite. The present study shows that also group I mGlu receptors may stimulate the formation of PAR. Our experiments performed in BHK cells transfected with group I mGlu receptors clearly demonstrate that activation of PARP can be triggered by stimulation of mGlu1 but not mGlu5 receptors. In cortical cell cultures, however, whereas the mGlu1 receptor antagonist 3-MATIDA completely abolished the DHPG-induced activation of PARP, the mGlu5 receptor antagonist MPEP was also able to reduce PARP activation, albeit to a considerably lesser degree. This finding suggests that, at least in cultured cortical neurons, PARP may be activated preferentially by mGlu1 receptors with a partial contribution of mGlu5 receptors. It is also possible that mGlu5 receptors may be activated indirectly by glutamate release following DHPG-induced depolarization.

It is not surprising that mGlu1 and mGlu5 receptors co-expressed in the same neuronal population may serve different physiological roles (Valenti et al., 2002). For example, in cultured cortical neurons stimulation of mGlu1 but not mGlu5 receptors induces potentiation of NMDA responses via a Ca^{2+}-calmodulin-dependent and protein kinase C-independent Pyk2/Src-family kinase pathway (Heidinger et al., 2002), while blockade of mGlu1 but not mGlu5 receptors is neuroprotective against OGD neuronal injury (Meli et al., 2002). The diversity of mGlu1 and mGlu5 receptor functions in these and other neurons can be explained by the tight and independent regulation of their specific coupling to downstream effectors or scaffolds, such as protein kinase C, G-protein-coupled receptor kinases (GRKs), regulators of G-protein signaling (RGS) proteins, and Homer and related proteins (Valenti et al., 2002).

Our finding that the preferential activation of mGlu1 receptors leads to PARP activation is particularly significant to the pathogenesis of cerebral ischemia. It has been demonstrated that mGlu1 rather than mGlu5 receptors play a distinct role in the pathways leading to post-ischemic neuronal death (Pellegrini-Giampietro, 2003). It has been proposed that the release of GABA and the subsequent activation of GABA receptors may contribute to the attenuation of post-ischemic injury induced by mGlu1 receptor antagonists (Battaglia et al., 2001; Cozzi et al., 2002), presumably via an



endocannabinoid retrograde signaling mechanism (Pellegrini-Giampietro et al., 2004). Our present experiments in a sublethal OGD model suggest that stimulation of mGlu1 receptor may lead to exacerbation of post-ischemic damage by an additional mechanism, i.e. the activation of PARP. A large body of evidence demonstrates that the increased neoformation of PAR plays a crucial role in neurodegeneration. In models of cerebral ischemia and excitotoxicity, it appears that intracellular depletion of ATP induced by PARP-1 leads to energy failure and necrotic neuronal death (Ha and Snyder, 1999; Meli et al., 2004; Moroni et al., 2001). In contrast, during the caspase-dependent apoptotic process, PARP-1 is proteolytically cleaved and inactivated by caspase-3, a process that is widely used as an apoptotic marker (Oliver et al., 1998). More recent studies have shown that PARP-1 may also have a role in caspase-independent apoptosis, by promoting the release of apoptosis-inducing factor (AIF) from mitochondria (Yu et al., 2002). Because, as all other available agents, the PARP inhibitor DPQ is not selective among members of the family (Southan and Szabo, 2003), it is not possible at present to identify the PARP subtype that is activated by mGlu1 receptor stimulation.

In our model of sublethal OGD, the PARP inhibitor DPQ was not able to reduce in a significant manner the release of LDH induced by the pseudo-ischemic insult. This finding may appear to be in contrast with previous results from our laboratory showing that PARP inhibitors are neuroprotective in cortical cell cultures exposed to OGD (Moroni et al., 2001). It should be noted, however, that OGD exposure in the present study was much milder (40 vs. 60 min) and that PARP inhibitors are known to be efficacious only when excitotoxic or ischemic insults are particularly intense and presumably necrotic (Bonfoco et al., 1995; Meli et al., 2004). In cultured cortical cells, DHPG by itself is not toxic but exacerbates LDH release and neuronal damage following exposure to OGD (Pellegrini-Giampietro et al., 1999a). Our results show that DPQ was able to completely abolish the component of OGD injury evoked by DHPG, strongly suggesting that PARP overactivation is responsible for the neuronal damage evoked by DHPG in this model.

Group I mGlu receptors are classically coupled to PLC-β, and therefore to the formation of IP_3 and diacylglycerol which results, respectively, in intracellular

Fig. 4. PAR immunocytochemistry in murine cortical cell cultures. Control cells (A) or cells treated with 500 μM H_2O_2 for 15 min (B), 100 μM DHPG for 60 min (C) or 100 μM DHPG + 100 μM 3-MATIDA for 60 min were incubated with a monoclonal anti-PAR antibody, exposed to a fluorescent secondary antibody and observed under fluorescence optics. H_2O_2 induced a dramatic increase in PAR immunoreactivity; a weaker but significant PAR signal was observed with DHPG that was prevented by the addition of 3-MATIDA. Scale bar: 50 μm.

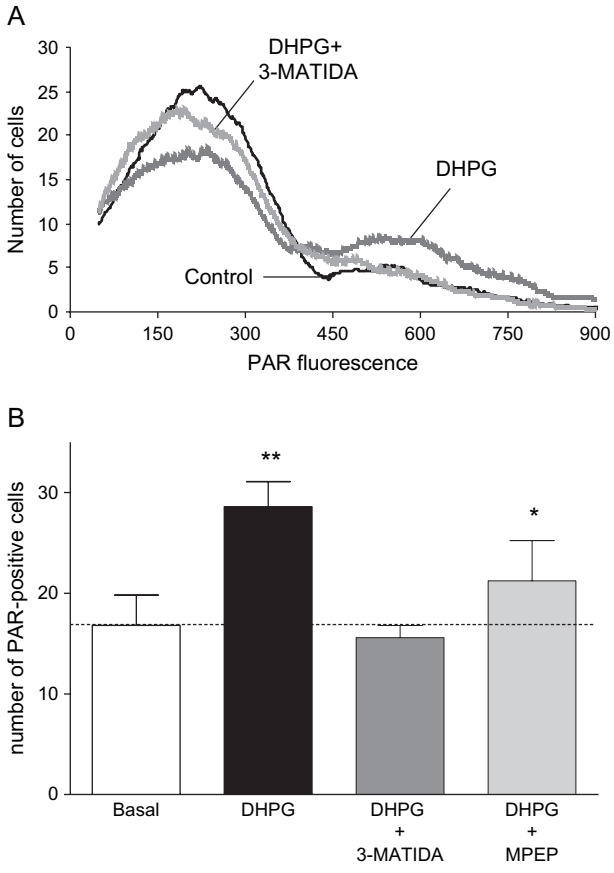


Fig. 5. PARP activation following DHPG exposure in murine cortical neurons. Cultures were exposed to 100 μM DHPG and after 60 min they were fixed, labeled with antibodies directed against PAR and GFAP, and processed for flow cytometry analysis. The GFAP-positive contribution to PAR fluorescence was subtracted from all samples. (A) The graph plots the relative number of cells displaying increasing levels of PAR fluorescence and is representative of five analyses. DHPG exposure elicited the formation of a peak at high levels of relative fluorescence intensity, indicative of the neosynthesis of PAR, that was reduced by 3-MATIDA (100 μM). (B) Percentage of PAR-positive cells in the entire neuronal population, as calculated by counting the relative number of neurons detected at channels for high levels of PAR fluorescence (>350). The DHPG-induced increase in PAR formation was completely reduced by 3-MATIDA (100 μM) and partly reduced by MPEP (1 μM). Results are the mean ± SEM of five experiments. $^{**}P < 0.01$ and $^{*}P < 0.05$ vs. untreated controls (ANOVA + Tukey's *w*-test).

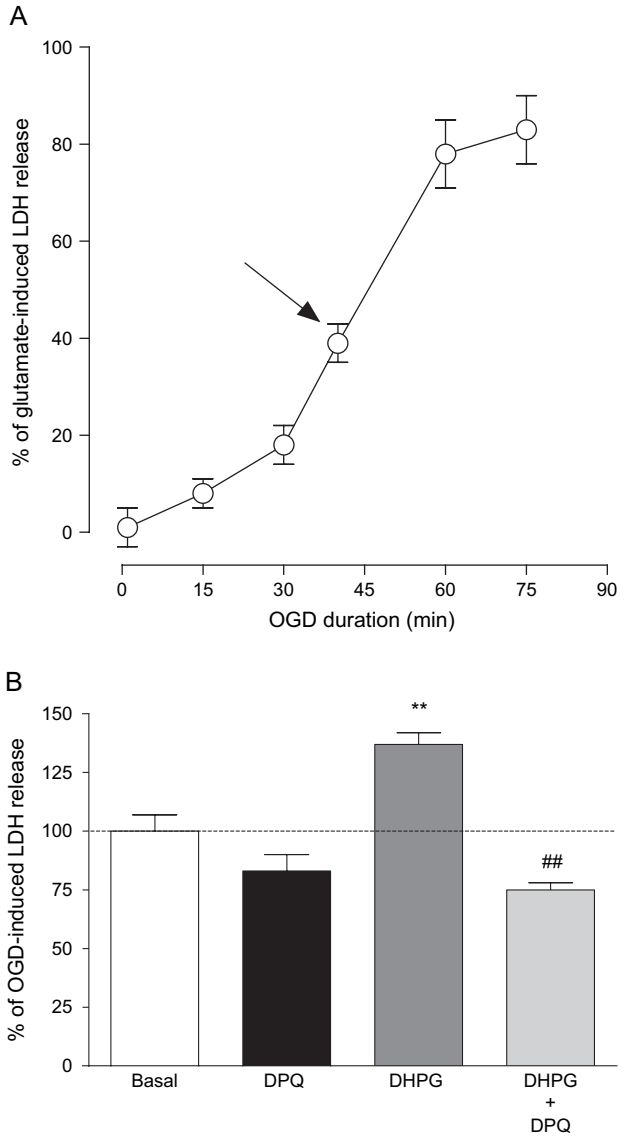


Fig. 6. The PARP inhibitor DPQ attenuates DHPG-induced neuronal damage in a model of sublethal OGD. (A) Time-dependent increase of neuronal death following different periods of OGD. Cells were exposed to OGD for the indicated period and neuronal death was assessed 24 h later by measuring LDH release in the medium. Data are expressed as percent of maximal damage, as produced by a 24-h exposure to 1 mM glutamate. For sublethal OGD experiments, a 40-min period was chosen (arrow). (B) Cultures were exposed to 40 min OGD and neuronal death was measured 24 h later. Data are expressed as percent of sublethal OGD-induced neuronal death. Application of 100 μM DHPG resulted in significant exacerbation of neuronal damage that was attenuated by co-administration of the PARP-1 inhibitor DPQ (10 μM). Values represent the mean ± SEM of at least five experiments. $^{**}P < 0.01$ vs. sublethal OGD and $^{\#\#}P < 0.01$ vs. DHPG (ANOVA + Tukey's *w*-test).

Ca^{2+} mobilization and activation of protein kinase C. mGlu1 and mGlu5 receptors are also linked to a variety of other effectors, such as adenylyl cyclase, tyrosine kinase, mitogen-activate protein kinase and phosphoinositide 3-kinase, that account for their diverse physiological and pathological effects (Bordi and Ugolini, 1999; Nicoletti et al., 1999; Valenti et al., 2002). Hence, it is not surprising that these receptors can couple to a pathway that leads to the activation of the nuclear enzyme PARP. It has been recently shown that high-$[K^+]$-induced membrane depolarization in cortical neurons promotes poly(ADP-rybosyl)ation in the absence of DNA breaks or NAD depletion (Homburg et al., 2000), a process that appears to be mediated by IP_3-induced intracellular Ca^{2+} release. Indeed, IP_3 production in depolarized neurons can be stimulated not only by phosphoinositide breakdown, but also by stimulation of tyrosine kinases or activation of trimeric

G-proteins (Berridge and Irvine, 1989; Exton, 1990). IP_3 receptors and IP_3-gated Ca^{2+} stores have been identified in the nuclear membrane (Gerasimenko et al., 1995). Group I mGlu receptors, and mGlu1 receptors in particular, may therefore trigger the nuclear formation of PAR via the production of IP_3, that then may act at the nuclear level to promote the activation of PARP and the formation of PAR.

In conclusion, PARP activation via mGlu1 receptor stimulation constitutes a novel pathway that may be of relevance in a number of physiological and pathological processes in neurons. For example, in view of the involvement of mGlu1 receptors in long-term potentiation and depression and the role of PARP in the regulation of gene expression and transcription, this mechanism may be operative in processes that are required for memory storage and consolidation (Cohen-Armon et al., 2004). In addition, our data suggest that the interplay between mGlu1 receptors and PARP may be responsible for the neuroprotective effects observed with mGlu1 receptor antagonists and PARP inhibitors in experimental models of cerebral ischemia.

Acknowledgements

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